

This listing of claims will replace all prior versions and listings of claims in the Application.

LISTING OF CLAIMS

1. **(Currently Amended)** A microfluidic apparatus for performing gel protein extractions, comprising:
 - a) an apparatus housing overlaid with an apparatus cover, wherein the housing has disposed therein a gel containing one or more proteins to be extracted and an electrolyte solution;
 - b) one or more fluidic channels containing an electrolyte solution, wherein the one or more fluidic channels have a first end and a second end, and wherein the first end is disposed through the apparatus cover and is secured in position at or near a gel interface;
 - c) one or more outlet reservoirs having disposed therein an electrolyte solution, a first end of ~~one or more~~ at least one outlet ~~electrodes~~ electrode, and the second end of the one or more fluidic channels; and
 - d) a high voltage power supply attached to a second end of the ~~one or more~~ at least one outlet electrode for applying an electric field across the length of the ~~on~~ one or more fluidic channels.
2. **(Currently Amended)** The apparatus of claim 1, wherein a ground electrode is connected to the apparatus ~~housing~~; housing.
3. **(Currently Amended)** The apparatus of claim 1, wherein each ~~channel~~ of the one or more fluidic channels is connected to the high voltage power supply through an array of switches allowing one or more individual fluidic channels to be selected for extraction independently from ~~the other~~ of the one or more fluidic channels.

4. **(Currently Amended)** The apparatus of claim 1, wherein ~~the first end of each of the one or more fluidic channels terminates in a fluidic separation channel, comprising~~ comprises:

a) one or more fluidic extraction channels containing an electrolyte solution, wherein the one or more fluidic extraction channels have a first end and a second end, and wherein the first end is disposed through the apparatus cover and is secured in position at the gel interface; and

b) one or more fluidic holding channels containing an electrolyte solution, wherein the one or more fluidic holding channels have a first end and a second end, and wherein the first end terminates in the one or more fluidic extraction channels, and wherein the second end of the one or more fluidic holding channels is disposed in the one or more outlet reservoirs.

~~e) one or more outlet reservoirs having disposed therein an electrolyte solution, a first end of one or more outlet electrodes, and the second end of the one or more fluidic holding channels;~~

~~d) a high voltage power supply connecting to the one or more outlet electrodes through an array of switches allowing one or more individual fluidic channels to be selected for extraction independently from the other fluidic channels.~~

5. **(Currently Amended)** The apparatus of claim 1, wherein a detector is near the one or more outlet reservoirs for monitoring the extracted proteins.

6. **(Original)** The apparatus of claim 1, wherein the one or more fluidic channels are capillaries.

7. **(Currently Amended)** The apparatus of claim 1, wherein the one or more fluidic channels are microscale channels.

8. **(Original)** The apparatus of claim 6, wherein the capillaries are fused silica capillaries.

9. ***(Currently Amended)*** The apparatus of claim 1, wherein the one or more fluidic channels are microfluidic channels formed in a planar glass substrate.
10. ***(Currently Amended)*** The apparatus of claim 1, wherein the one or more fluidic channels are microfluidic channels formed in a planar plastic substrate.
11. ***(Currently Amended)*** The apparatus of claim 1, wherein the one or more fluidic channels are of a diameter which allows them to extract the one or more proteins in the gel in about two minutes or less.
12. ***(Currently Amended)*** The apparatus of claim 1, wherein the one or more fluidic channels are of a diameter which allows them to extract the one or more proteins in the gel in about ten minutes or less.
13. ***(Currently Amended)*** The apparatus of claim 1, wherein the at least one outlet electrode is constructed of platinum.
14. ***(Currently Amended)*** The apparatus of claim 1, wherein the at least one outlet electrode is constructed of gold.
15. ***(Currently Amended)*** The apparatus of claim 1, wherein the at least one outlet electrode is a thin film metal integrated into a glass substrate.
16. ***(Currently Amended)*** The apparatus of claim 1, wherein the at least one outlet electrode is a thin film metal integrated into a plastic substrate.

17. **(Original)** The apparatus of claim 6, wherein the capillaries have an outer diameter of between about 100 μm and about 500 μm and an inner diameter of between about 5 μm and about 100 μm .

18. **(Original)** The apparatus of claim 6, wherein the capillaries are between about 1 cm and about 50 cm long.

19. **(Currently Amended)** The apparatus of claim 1, wherein the electric field across the one or more fluidic channels is between about 100 V/cm and about 1000 V/cm.

20. **(Original)** The apparatus of claim 1, wherein the apparatus cover and the apparatus housing create a gel chamber compartment that is pressurizable.

21. **(Currently Amended)** A method of transferring one or more proteins from a gel, comprising:

a) contacting a first end of one or more fluidic channels containing an electrolyte solution to one or more locations in ~~the~~ a gel containing ~~the~~ one or more proteins, wherein:

1) the gel is disposed within an apparatus housing overlaid with an apparatus cover and the housing has disposed therein an electrolyte solution and has attached thereto a ground electrode;

2) the one or more fluidic channels have a first end and a second end;

3) the first end of the one or more fluidic channels is disposed through the apparatus cover and is secured in position at or near ~~the~~ a gel interface;

b) disposing the second end of the one or more fluidic channels in one or more outlet 20 reservoirs, wherein:

1) the one or more outlet ~~reservoir~~ reservoirs have disposed therein an electrolyte solution and a first end of an outlet electrode; and

2) a high voltage power supply is attached to the outlet electrode;

c) applying a high electric field along the length of the one or more fluidic channels, thereby extracting the one or more proteins from the gel and into the first end of the one or more fluidic channels;

d) concentrating the one or more proteins near the first end of the one or more fluidic channels by electrophoretic ~~stacking~~, stacking, and

e) transferring the one or more proteins from the first end of the one or more fluidic channels toward the second end of the one or more fluidic channels.

22. ***(Currently Amended)*** The method of claim 21, wherein the one or more proteins are transferred from the first end of the one or more fluidic channels toward the second end of the one or more fluidic channels in step ~~(d)~~ (e) by a method further comprising:

a) stopping the high electric field across the one or more fluidic channels;

b) removing the first end of the one or more fluidic channels from the gel interface and transferring the first end of the one or more fluidic channels into a reservoir of fresh electrolyte solution; and

c) reapplying the high electric field to the one or more fluidic channels so that the one or more proteins are transferred from the first end of the one or more fluidic channels toward the second end of the one or more fluidic channels for at least one of analysis ~~and/or~~ or collection.

23. **(Currently Amended)** The method of claim 21, wherein the one or more proteins are transferred from the first end of the one or more fluidic channels toward the second end of the one or more fluidic channels in step ~~(d)~~ (e) by a method further comprising:

- a) stopping the high electric field across the one or more fluidic channels;
- b) raising the one or more fluidic channels slightly above the gel interface; and
- c) pressurizing ~~the~~ a compartment created by the apparatus housing and the apparatus cover so that the one or more proteins are transferred from the first end of the one or more fluidic channels toward the second end of the one or more fluidic channels for at least one of collection and/or or analysis.

24. **(Currently Amended)** The method of claim 21, wherein the one or more proteins are transferred from the first end of the one or more fluidic channels toward the second end of the one or more fluidic channels in step ~~(d)~~ (e) by a method further comprising allowing the high electric field to continually transfer the one or more proteins from the first end of the one or more fluidic channels to the second end of the one or more fluidic channels while the first end of the one or more fluidic channels is still contacted to the gel.

25. **(Currently Amended)** The method of claim 21, wherein the one or more proteins are transferred from the first end of the one or more fluidic channels toward the second end of the one or more ~~microscale~~ fluidic channels in step ~~(d)~~ (e) by a method further comprising:

- a) stopping the high electric field across the one or more ~~microscale~~ fluidic channels;
- b) raising the one or more ~~microscale~~ fluidic channels slightly above the gel interface;
and
- c) applying a negative pressure at the second end of the one or more fluidic channels relative to ~~the~~ a compartment pressure within the apparatus housing so that the one or more

proteins are transferred from the first end of the one or more fluidic channels toward the second end of the one or more fluidic channels for at least one of collection ~~and/or~~ or analysis.

26. **(Currently Amended)** The method of claim 21, wherein one or more instruments are located on or near the one or more fluidic channels to analyze the one or more proteins.

27. **(Currently Amended)** The method of claim 26, wherein the ~~instrument is~~ one or more instruments are a UV detector or a fluorescence detector.

28. **(Original)** The method of claim 21, wherein the one or more proteins are subject to digestion.

29. **(Currently Amended)** The method of claim 28, wherein the one or more ~~digested~~ proteins are subject to digestion ~~digested~~ before transfer to the one or more fluidic channels.

30. **(Currently Amended)** The method of claim 29, wherein ~~the one or more digested proteins~~ undergo digestion ~~includes~~ in-gel digestion prior to transfer.

31. **(Currently Amended)** The method of claim 29, wherein the one or more digested proteins are fluidically transferred into a mass spectrometer from the first end of the ~~microfluidic~~ one or more fluidic channels.

32. **(Currently Amended)** The method of claim 29, wherein the one or more digested proteins are fluidically transferred into a mass spectrometer from the second end of the ~~microfluidic~~ one or more fluidic channels.

33. *(Currently Amended)* The method of claim 29, wherein the one or more digested proteins are fluidically transferred from the first end of the ~~microfluidic~~ one or more fluidic channels onto a MALDI target plate for further analysis by a mass spectrometer.

34. *(Currently Amended)* The method of claim 29, wherein the one or more digested proteins are fluidically transferred from the second end of the ~~microfluidic~~ one or more fluidic channels onto a MALDI target plate for further analysis by a mass spectrometer.

35. *(Canceled)*

36. *(Currently Amended)* The method of claim 28, wherein the one or more ~~digested~~ proteins are ~~digested~~ subject to digestion during transfer to the one or more fluidic channels.

37. *(Currently Amended)* The method of claim 36, wherein the one or more proteins undergo digestion in a membrane containing immobilized proteolytic enzymes positioned between the gel and the first end of the one or more fluidic channels.

38. *(Currently Amended)* The method of claim 36, wherein the one or more digested proteins are fluidically transferred into a mass spectrometer from the first end of the ~~microfluidic~~ one or more fluidic channels.

39. *(Currently Amended)* The method of claim 36, wherein the one or more digested proteins are fluidically transferred into a mass spectrometer from the second end of the ~~microfluidic~~ one or more fluidic channels.

40. *(Currently Amended)* The method of claim 36, wherein the one or more digested proteins are fluidically transferred from the first end of the ~~microfluidic~~ one or more fluidic channels onto a MALDI target plate for further analysis by a mass spectrometer.

41. *(Currently Amended)* The method of claim 36, wherein the one or more digested proteins are fluidically transferred from the second end of the ~~microfluidic~~ one or more fluidic channels onto a MALDI target plate for further analysis by a mass spectrometer.

42. *(Cancelled)*

43. *(Currently Amended)* The method of claim 28, wherein the one or more ~~digested~~ proteins are ~~digested~~ subject to digestion after transfer to the one or more fluidic channels.

44. *(Currently Amended)* The method of claim 43, wherein the one or more proteins ~~is~~ are transferred directly from the one or more fluidic channels to a micro membrane reactor containing proteolytic enzymes for digestion.

45. *(Currently Amended)* The method of claim 43, wherein the one or more proteins ~~is~~ are transferred directly from the one or more fluidic channels to a column reactor containing particles or beads immobilized with proteolytic enzymes for digestion.

46. *(Currently Amended)* The method of claim 43, wherein the one or more fluidic channels ~~contains~~ contain particles or beads immobilized with proteolytic enzymes for digestion.

47. **(Currently Amended)** The method of claim 43, wherein proteolytic enzymes for protein digestion are contained in solution within the one or more fluidic channels.
48. **(Currently Amended)** The method of claim 43, wherein the one or more digested proteins are fluidically transferred into a mass ~~Spectrometer~~ spectrometer from the first end of the ~~microfluidic~~ one or more fluidic channels.
49. **(Currently Amended)** The method of claim 43, wherein the one or more digested proteins are fluidically transferred into a mass spectrometer from the second end of the ~~microfluidic~~ one or more fluidic channels.
50. **(Currently Amended)** The method of claim 43, wherein the one or more digested proteins are fluidically transferred from the first end of the ~~microfluidic~~ one or more fluidic channels onto a MALDI target plate for further analysis by a mass spectrometer.
51. **(Currently Amended)** The method of claim 43, wherein the one or more digested proteins are fluidically transferred from the second end of the ~~microfluidic~~ one or more fluidic channels onto a MALDI target plate for further analysis by a mass spectrometer.
52. **(Cancelled)**
53. **(Original)** The method of claim 21, wherein the one or more proteins are denatured in sodium dodecyl sulfate.

54. **(Currently Amended)** The method of claim 21, wherein the one or more fluidic channels are capillaries.

55. **(Currently Amended)** The method of claim 21, wherein the one or more fluidic channels are microfluidic channels formed in a planar glass substrate.

56. **(Currently Amended)** The method of claim 21, wherein the one or more fluidic channels are microfluidic channels formed in a planar plastic substrate.

57. **(Currently Amended)** The method of claim 21, wherein the ~~capillaries~~ one or more fluidic channels are coated with hydrophilic polymers ~~such as polyacrylamide~~.

58. **(Currently Amended)** The method of claim 21, wherein a ~~plurality of~~ the one or more fluidic channels are arranged in an array, and the array contacts the gel.

59. **(Currently Amended)** The method of claim 21, wherein the high electric field within each of the one or more fluidic channels is individually addressable for extraction independently from ~~the other~~ of the one or more fluidic channels.

60. **(Currently Amended)** The method of claim 21, wherein the ~~plurality of~~ one or more fluidic channels may be positioned sequentially or simultaneously at various gel locations using a manual or automated positioning system, enabling individual or groups of the one or more fluidic channels within the array to sequentially or simultaneously extract multiple proteins from the various gel locations using a single extraction apparatus.

61. **(Original)** The method of claim 21, wherein the electrolyte solution contains Tris-HC 1 at a concentration of at most about 25 mM Tris-HCL at about pH 6.8.
62. **(Original)** The method of claim 21, wherein the gel is made of polyacrylamide or agarose.
63. **(Original)** The method of claim 21, wherein the gel is between about 1 mm and about 100 μ m thick.
64. **(Original)** The method of claim 21, wherein the gel is a gradient gel in the range of about 4% to about 20% polyacrylamide.
65. **(Original)** The method of claim 21, wherein the gel is a Tris/Tricine SDS polyacrylamide gel.
66. **(Original)** The method of claim 21, wherein the gel was used to perform 1D or 2D gel electrophoresis.
67. **(Currently Amended)** The method of claim 21, wherein the locations to place the first end of the one or more fluidic channels are visualized or imaged.
68. **(Original)** The method of claim 67, wherein the visualization is performed with Coomassie blue.

69. *(Currently Amended)* The method of claim 67, wherein the visualization is performed with silver staining.
70. *(Original)* The method of claim 67, wherein the visualization is performed with SYPRO fluorescent dyes.
71. *(New)* The method of claim 30, wherein the digestion is performed with a proteolytic enzyme.
72. *(New)* The method of claim 71, wherein the proteolytic enzyme is trypsin.
73. *(New)* The method of claim 37, wherein the proteolytic enzymes include trypsin.
74. *(New)* The method of claim 44, wherein the proteolytic enzymes include trypsin.
75. *(New)* The method of claim 45, wherein the proteolytic enzymes include trypsin.
76. *(New)* The method of claim 46, wherein the proteolytic enzymes include trypsin.
77. *(New)* The method of claim 47, wherein the proteolytic enzymes include trypsin.
78. *(New)* The method of claim 57, wherein the hydrophilic polymers include polyacrylamide.